Cancer Immunology, Immunotherapy (2026) 75:182
https://doi.org/10.1007/s00262-026-04415-x

RESEARCH

NY-ESO-1 and PD-L1 as biomarkers associated with nivolumab response and outcome in unresectable or recurrent esophageal squamous cell carcinoma: a multicenter biomarker study

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Received: 21 March 2026 / Accepted: 4 May 2026

Abstract

Background Programmed cell death protein-1 (PD-1) blockade improves survival in esophageal squamous cell carcinoma (ESCC), although only a subset of patients respond. We aimed to identify tumor-related biomarkers associated with response and outcome in patients with unresectable or recurrent ESCC.

Methods This multicenter retrospective study included 250 patients with unresectable or recurrent ESCC who received nivolumab as second- or later-line therapy. Pretreatment tumor specimens were evaluated by immunohistochemistry for p53, NY-ESO-1, MLH1, and programmed death-ligand 1 (PD-L1). PD-L1 expression was assessed using both tumor proportion score (TPS) and combined positive score (CPS) by using automated digital image analysis. Associations with objective response, progression-free survival (PFS), and overall survival (OS) were analyzed.

Results NY-ESO-1 expression was significantly associated with response to nivolumab (28.6% vs. 11.1%, $P=0.005$) and remained independently associated with response in multivariate analysis (OR 3.32, 95% CI 1.49–7.28, $P=0.0027$). PD-L1 expression was also associated with response according to both CPS and TPS. Patients with CPS $\geq$ 10% showed higher response rates than those with CPS $<$ 10% (24.1% vs. 10.2%, $P=0.003$), as well as longer median PFS (2.6 vs. 2.0 months, $P=0.0173$) and OS (12.3 vs. 10.4 months, $P=0.0479$). In contrast, p53 expression showed no significant association with response or survival, and MLH1 loss was rare.

Conclusions NY-ESO-1 expression was associated with response to nivolumab, while PD-L1 expression, particularly CPS, may provide clinically relevant prognostic information. Integrated assessment of tumor-related biomarkers and the host immune microenvironment may further improve patient stratification in ESCC.

Keywords Anti-PD-1 antibody · Biomarker · Esophageal squamous cell carcinoma · NY-ESO-1 · PD-L1

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Published online: 12 May 2026

Introduction

Esophageal cancer remains one of the leading causes of cancer-related death worldwide, with 511,054 new cases and 445,391 deaths reported in 2022 [1]. Esophageal squamous cell carcinoma (ESCC) accounts for approximately 85% of esophageal cancers globally [2]. Despite advances in multidisciplinary treatment, many patients are diagnosed at an advanced stage, and recurrence after curative-intent therapy remains common [3–10]. As a result, the prognosis of patients with unresectable or recurrent ESCC remains poor, highlighting the need for improved therapeutic strategies [4, 5, 9–11].

Immune checkpoint inhibitors targeting the programmed cell death protein-1 (PD-1) pathway have become an important treatment option for advanced ESCC. By blocking PD-1–mediated suppression of T-cell activation, these agents enhance antitumor immunity [12]. In the ATTRACTION-3 trial, nivolumab demonstrated superior overall survival compared with taxane chemotherapy in patients with unresectable advanced or recurrent ESCC, with durable benefits observed at long-term follow-up [13, 14]. However, only a minority of patients respond to nivolumab, with objective response rates of approximately 17–19% [10, 13]. Therefore, biomarkers capable of predicting benefit from PD-1 blockade are needed.

Several tumor-related biomarkers have been investigated as potential predictors of response to immune checkpoint inhibitors, including programmed death-ligand 1 (PD-L1) expression, mismatch repair (MMR) deficiency, cancer-testis antigens, and tumor suppressor pathway alterations [15–22]. Among these, PD-L1 expression is currently the most widely used biomarker in ESCC, although its predictive value remains limited [17, 18]. Other candidates such as the cancer-testis antigen NY-ESO-1 and p53 alterations have also been suggested to influence antitumor immunity and responses to immunotherapy [21–24]. However, the clinical significance of these tumor-related factors in patients with unresectable or recurrent ESCC treated with nivolumab remains unclear. PD-L1, NY-ESO-1, p53, and MLH1 were selected because they represent clinically and biologically relevant tumor-related features potentially associated with response to PD-1 blockade in ESCC.

We recently reported clinical outcomes of nivolumab treatment in a large multi-institutional cohort of patients with unresectable or recurrent ESCC [10] and demonstrated the importance of host immune microenvironment factors such as tumor-infiltrating lymphocytes and tertiary lymphoid structures [25]. Building on these findings, the present study aimed to evaluate tumor-related biomarkers associated with nivolumab efficacy and survival in this cohort. Specifically, we assessed PD-L1, NY-ESO-1, p53, and MLH1 expression in pretreatment tumor samples and examined their associations with treatment outcomes and the local immune microenvironment.

Material and methods

Patients

This multicenter retrospective study included 250 patients with unresectable or recurrent esophageal squamous cell carcinoma (ESCC) who received nivolumab as second-line or later therapy between 2014 and 2022 at 15 institutions participating in the Clinical Study Group of Osaka University, Upper Gastrointestinal Surgery Group. Patients were eligible if they had histologically confirmed ESCC refractory or intolerant to at least one prior chemotherapy regimen, were aged ≥ 20 years, and had available pretreatment tumor tissue samples from the primary lesion containing residual tumor cells. Patients previously treated with immune checkpoint inhibitors other than nivolumab were excluded. Patients with synchronous or metachronous malignancies diagnosed within 5 years prior to nivolumab initiation were also excluded, except for carcinoma in situ or mucosal carcinoma. Written informed consent was obtained from all included patients whenever possible; consent was waived for patients who had died or were lost to follow-up. The study was approved by the institutional review boards of all participating institutions (approval no. 19146-5) and was registered in the UMIN Clinical Trials Registry (UMIN000040462).

Evaluations of tumor response

Although follow-up schedules were not standardized across institutions, treatment efficacy was generally evaluated every 6–8 weeks. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [26]. A minimum interval of 6 weeks between imaging assessments was required to determine complete response (CR), partial response (PR), or stable disease (SD) [27, 28]. Response rate was evaluated only in patients with measurable lesions. Treatment response was defined as the proportion of patients who achieved a best overall response of CR or PR.

Immunohistochemistry for PD-L1, p53, NY-ESO-1, and MLH1

Archival primary tumor specimens used for immunohistochemical analyses were obtained either from diagnostic endoscopic biopsy or from surgically resected specimens.

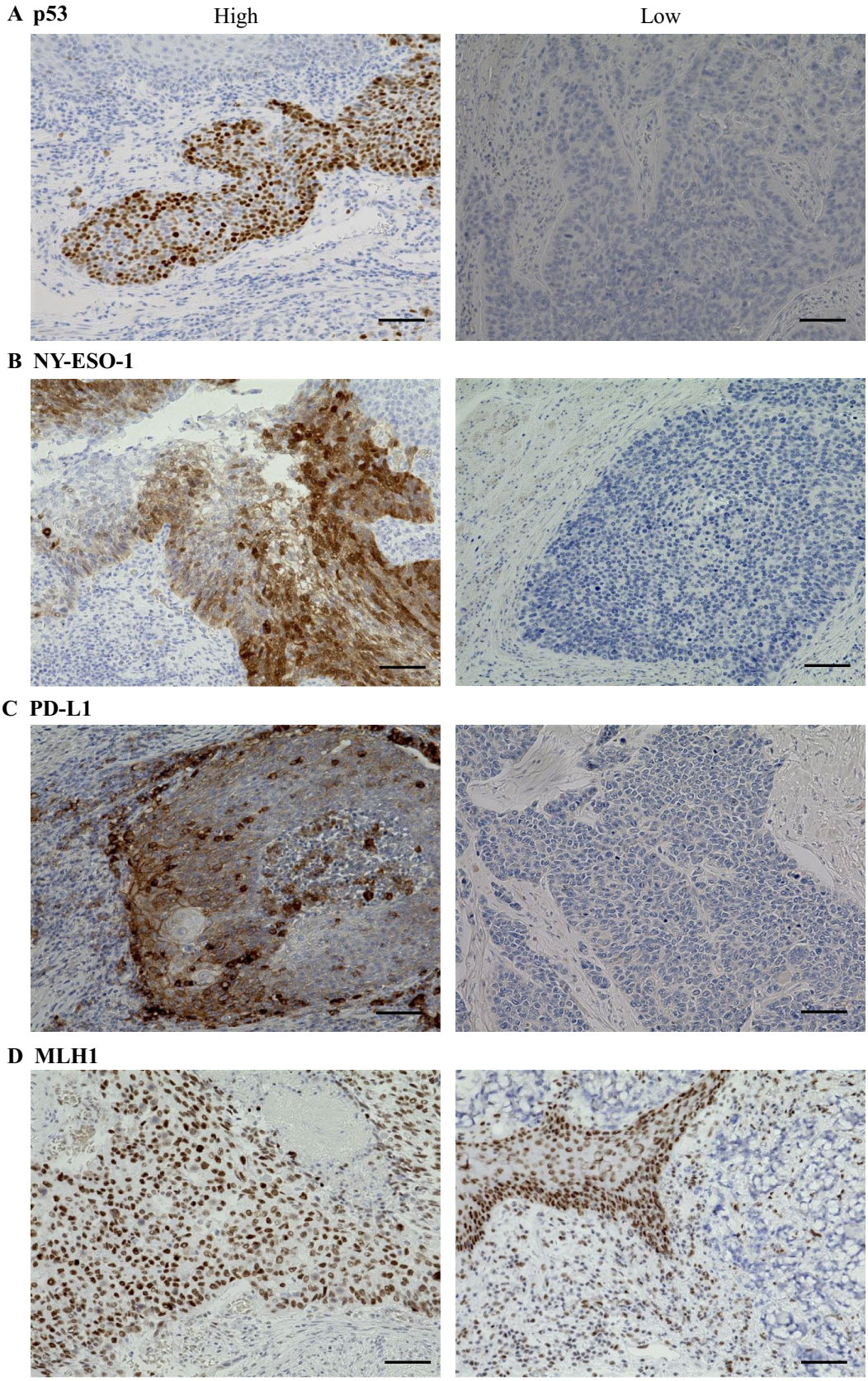


Fig. 1 Representative immunohistochemical images of p53, NY-ESO-1, PD-L1, and MLH1 expression in pretreatment ESCC specimens. **A** p53. **B** NY-ESO-1. **C** PD-L1. **D** MLH1. Representative examples of high and low/negative expression are shown for each marker. Scale bars: 200 μm

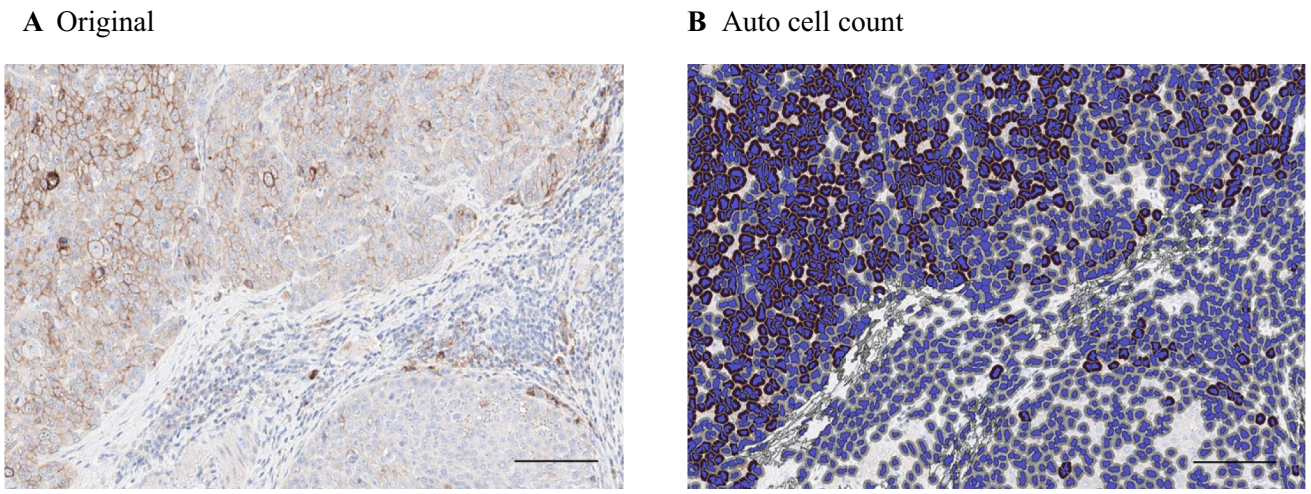


Fig. 2 Automated PD-L1 scoring using HALO software. **A** Original image. **B** Representative image showing automated cell detection and quantification used to calculate the tumor proportion score (TPS) and combined positive score (CPS). Tumor cells and mononuclear immune cells were distinguished morphologically by the observers, and PD-L1–positive cells were identified and counted using HALO image analysis software. Representative examples of the morphology-based distinction between tumor cells and mononuclear immune cells are shown in Supplementary Figure S2. PD-L1–positive cells were evaluated in five hotspot fields at 400 × magnification. Scale bars: 100 µm

In patients with unresectable disease, diagnostic biopsy specimens obtained at initial diagnosis were analyzed [29]. In patients with recurrent disease, archival surgical specimens from the initial treatment were used, because contemporaneous biopsy samples at the time of nivolumab initiation were not uniformly available in this retrospective multicenter cohort. Therefore, biopsy and surgical specimens were mutually exclusive.

All samples were fixed in 10% neutral-buffered formalin for 24–72 h and embedded in paraffin. Pathologists blinded to clinical information selected formalin-fixed paraffin-embedded (FFPE) blocks containing representative tumor regions, including the deepest invasive area, and these samples were centrally analyzed. FFPE tissues were cut into 4-µm sections and subjected to immunohistochemical staining using the Dako Autostainer Link 48 system (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Primary antibodies were incubated for 30 min at room temperature. The specificity of each antibody was confirmed using human tonsil tissue as a positive control (Supplementary Figure S1). These four markers were selected a priori to examine distinct tumor-related biological features potentially relevant to PD-1 blockade in ESCC. The following primary antibodies were used: PD-L1 (clone 28-8, ready-to-use; Agilent Technologies), p53 (DO-7, 1:500; Novocastra/Leica Biosystems, Newcastle upon Tyne, UK), NY-ESO-1 (E978, 1:100; Santa Cruz Biotechnology, Dallas, TX, USA), and MLH1 (ES05, 1:50; Novocastra/Leica Biosystems, Newcastle upon Tyne, UK).

Evaluation of p53, NY-ESO-1, and MLH1 expressions

All stained slides were digitized using a Ventana iScan HT scanner (Roche Diagnostics, Sant Cugat, Spain) to generate 20 × whole-slide images. Two independent observers (S.N. and T.M.), blinded to clinicopathological data, manually defined tumor regions on each slide, and discrepancies were resolved by consensus. Whole-slide images were analyzed using HALO image analysis software (Indica Labs, Corrales, NM, USA). The software automatically detected and quantified positively stained tumor cells within the annotated tumor area. For p53 and NY-ESO-1, positivity was defined as > 10% nuclear staining and > 5% stained tumor cells, respectively, whereas staining intensity was not incorporated into the scoring. Complete absence of p53 staining (null pattern) was not classified separately as abnormal and was included in the negative group. MLH1 loss was defined as complete absence of nuclear staining in tumor cells (Fig. 1). These thresholds were predefined on the basis of previous reports and applied uniformly across the cohort (Fig. 1).

Evaluation of PD-L1 expression

PD-L1 expression was evaluated using both the tumor proportion score (TPS) and the combined positive score (CPS). The PD-L1 clone 28—8 assay was selected because it was used in major nivolumab clinical trials in ESCC and has been clinically validated for PD-L1 assessment in this disease [30]. Whole-slide images were reviewed in

Table 1 Association between tumor-related biomarker expression and response to nivolumab

		Responders (n = 36)	Non-responders (n = 214)	*P*
p53	Positive	22 (13.3%)	146 (86.8%)	0.400
	Negative	14 (17.3%)	68 (82.7%)	
NY-ESO-1	Positive	14 (28.6%)	35 (71.4%)	0.005
	Negative	22 (11.1%)	179 (88.9%)	
MLH1	Positive	35 (14.1%)	214 (85.9%)	0.934
	Negative	1 (100%)	0 (0%)	
PD-L1	CPS ≥ 10%	19 (24.1%)	61 (75.9%)	0.003
	CPS < 10%	17 (10.2%)	150 (89.8%)	
	TPS ≥ 1%	24 (20.3%)	95 (79.7%)	0.018
	TPS < 1%	12 (9.4%)	116 (90.6%)	

PD-L1 programmed death-ligand 1, *TPS* tumor proportion score, *CPS* combined positive score

five hotspot regions at 400 × magnification. For TPS and CPS assessment, tumor cells and mononuclear immune cells were distinguished morphologically by the observers, whereas PD-L1-positive cells within the selected fields were identified and counted using HALO image analysis software (Indica Labs). A representative image used for TPS and CPS assessment is shown in Fig. 2, and representative examples of the morphology-based distinction between tumor cells and mononuclear immune cells are shown in Supplementary Figure S2. TPS was defined as the percentage of PD-L1–positive tumor cells among all viable tumor cells. CPS was calculated as the number of PD-L1–positive cells, including tumor cells and mononuclear immune cells, divided by the total number of viable tumor cells and multiplied by 100. Patients were categorized using predefined cutoffs of TPS ≥ 1% and CPS ≥ 10% for subsequent analyses. Associations between PD-L1 expression and nivolumab response and survival were then evaluated. Hotspot selection was standardized by review of the entire available tumor area on each whole-slide image, and in biopsy specimens hotspot regions were selected from the available evaluable tumor-containing area on the slide.

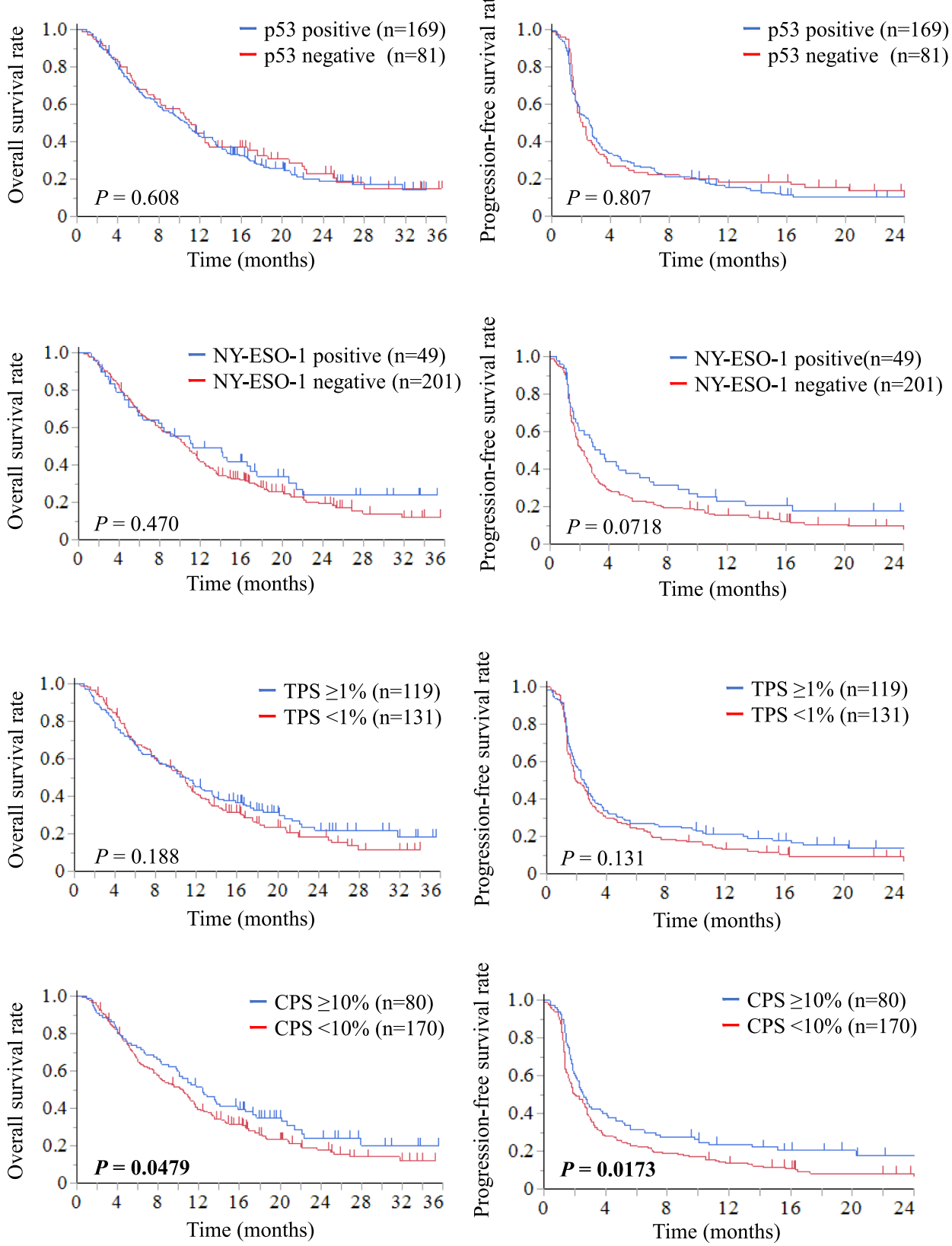
Assessment of previously evaluated host immune microenvironment factors

For the combined analyses of tumor-related and host immune microenvironment factors, previously evaluated data on CD8 + cell infiltration and tertiary lymphoid structure (TLS) density in surgical specimens from the same cohort were used, as reported previously [25, 31]. CD8 + cell infiltration was used as an indicator of pre-existing effector antitumor immunity, whereas TLS density was included as a marker of local adaptive immune organization within the tumor microenvironment. Briefly, CD8 + cells in surgical specimens were quantified in the 1000-μm peritumoral margin using HALO-based digital image analysis, and TLS density was assessed on hematoxylin and eosin-stained sections in the peritumoral area, as previously described. Representative images of CD8 + cell infiltration and tertiary lymphoid structures (TLSs), together with a schematic summary of their quantification methods, are shown in Supplementary Figure S3.

Table 2 Univariate and multivariate analyses of factors associated with response to nivolumab

Variables	Category	Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Age (y)	≥ 70	1.43 (0.69–2.91)	0.3301		
Sex	Female	1.33 (0.61–3.03)	0.4429		
Performance status	1–3	0.61 (0.30–1.26)	0.1825		
History of smoking	Yes	0.92 (0.42–2.03)	0.8394		
Previous surgery	No	0.78 (0.38–1.60)	0.5053		
Previous radiotherapy	Yes	0.95 (0.46–1.95)	0.8939		
Number of previous chemotherapy regimens	3-	0.68 (0.23–2.06)	0.5027		
Number of organs with metastases	3-	0.39 (0.11–1.34)	0.1359		
p53	Yes	0.71 (0.34–1.49)	0.3700		
NY-ESO-1	Positive	3.25 (1.49–6.93)	**0.0033**	3.32 (1.49–7.28)	**0.0027**
TPS	≥ 1%	2.50 (1.21–5.42)	**0.0128**	1.43 (0.51–3.88)	0.4828
CPS	≥ 10%	2.80 (1.33–5.80)	**0.0051**	2.29 (0.88–6.37)	0.0865

CI confidence interval, *OR* odds ratio, *PD-L1* programmed death-ligand 1, *TPS* tumor proportion score, *CPS* combined positive score


Overall survival rate
p53 positive (n=169)
p53 negative (n=81)
P = 0.608
Time (months)
Progression-free survival rate
p53 positive (n=169)
p53 negative (n=81)
P = 0.807
Time (months)
Overall survival rate
NY-ESO-1 positive (n=49)
NY-ESO-1 negative (n=201)
P = 0.470
Time (months)
Progression-free survival rate
NY-ESO-1 positive(n=49)
NY-ESO-1 negative (n=201)
P = 0.0718
Time (months)
Overall survival rate
TPS ≥1% (n=119)
TPS <1% (n=131)
P = 0.188
Time (months)
Progression-free survival rate
TPS ≥1% (n=119)
TPS <1% (n=131)
P = 0.131
Time (months)
Overall survival rate
CPS ≥10% (n=80)
CPS <10% (n=170)
P = 0.0479
Time (months)
Progression-free survival rate
CPS ≥10% (n=80)
CPS <10% (n=170)
P = 0.0173
Time (months)

◂**Fig. 3** Kaplan–Meier curves for overall survival (OS) and progression-free survival (PFS) according to biomarker expression in the overall cohort. OS and PFS are shown according to p53 expression, NY-ESO-1 expression, PD-L1 TPS, and PD-L1 CPS. The objective response rate in the entire cohort was 14.4% (36/250). Patients with CPS ≥ 10% showed significantly better PFS and OS than those with CPS < 10%. No significant differences in OS or PFS were observed according to p53 expression or TPS status. NY-ESO-1–positive tumors showed a trend toward improved PFS, although the difference did not reach statistical significance. This cohort was previously reported in our biomarker study (PMID: 40274705)

Statistical analysis

Continuous variables are presented as medians with ranges and were compared using the Mann–Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Survival outcomes were analyzed using the Kaplan–Meier method and compared using the log-rank test. Progression-free survival (PFS) was defined as the interval from the first administration of nivolumab to disease progression or death from any cause. Overall survival (OS) was defined as the interval from the first nivolumab administration to death from any cause. The Cox proportional hazards model was used to estimate hazard ratios (HRs) and perform univariate and multivariate analyses for PFS. Variables that were significant in univariate analysis were included in multivariate models. Logistic regression analysis was performed to identify factors associated with response to nivolumab, and odds ratios (ORs) were calculated. Continuous CPS values were not included in the prespecified statistical analyses, and CPS was analyzed categorically using the predefined cutoff of 10. All statistical tests were two-sided, and P values < 0.05 were considered statistically significant. Statistical analyses were conducted using JMP Pro software (version 16.2.0; SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics and treatment outcomes

Baseline characteristics of the study cohort are summarized in Supplementary Table S1. The study included 250 patients with unresectable or recurrent ESCC who received nivolumab. The median age at nivolumab initiation was 70 years (range, 32–89 years), and 193 patients (77.2%) were male. An Eastern Cooperative Oncology Group performance status (PS) of 0 was observed in 120 patients (48.0%). Regarding disease status, 131 patients (52.4%) had unresectable advanced disease and 119 (47.6%) had recurrent disease. All patients had received prior systemic chemotherapy. Previous surgery had been performed in 133 patients (53.2%), and 107 patients (42.8%) had received prior radiotherapy. Pretreatment tumor specimens consisted of endoscopic biopsy samples in 121 patients (48.4%) and surgical specimens in 129 patients (51.6%). Best overall responses to nivolumab were complete response (CR) in 5 patients (2.0%), partial response (PR) in 31 (12.4%), stable disease (SD) in 80 (32.0%), and progressive disease (PD) in 134 (53.6%). The objective response rate was therefore 14.4% (36/250), and the disease control rate was 46.4% (116/250).

Expressions of p53, NY-ESO-1, MLH1, and PD-L1 in pretreatment samples

The expression status of tumor-related biomarkers is also summarized in Supplementary Table S2. Among the 250 tumors, p53 expression was positive in 169 cases (67.6%) and negative in 81 (32.4%). NY-ESO-1 expression was detected in 49 tumors (19.6%), whereas 201 tumors (80.4%) were negative. MLH1 expression was retained in almost all tumors (249/250, 99.6%), with loss of expression observed in only one case (0.4%). For PD-L1 expression, a tumor proportion score (TPS) ≥ 1% was observed in 119 cases (47.6%), while 131 cases (52.4%) had TPS < 1%. In addition, 80 tumors (32.0%) showed a combined positive score (CPS) ≥ 10%, whereas 170 tumors (68.0%) had CPS < 10%. NY-ESO-1 positivity did not significantly differ between biopsy and surgical specimens (26/121 [21.5%] vs. 23/129 [17.8%], $P = 0.466$).

To examine whether intervening treatment exposure differed according to NY-ESO-1 status, we additionally assessed treatment exposure between tissue acquisition and nivolumab initiation (Supplementary Table S3). Treatment exposure patterns—chemotherapy alone, (chemo-)radiation alone, and chemotherapy plus (chemo-)radiation—did not significantly differ between NY-ESO-1–positive and NY-ESO-1–negative tumors (27/49 [55.1%], 7/49 [14.3%], and 15/49 [30.6%] vs. 116/201 [57.7%], 35/201 [17.4%], and 50/201 [24.9%], respectively; $P = 0.6824$).

Association between NY-ESO-1 expression and host immune microenvironment

To investigate the relationship between tumor-related biomarkers and the host immune microenvironment, we examined the associations between NY-ESO-1 expression and previously evaluated immune parameters in surgical specimens [25]. As shown in **Supplementary Table S4**, NY-ESO-1 expression was not significantly associated with clinicopathological factors including age, sex, performance status, BMI, smoking history, tumor status, number of metastatic organs, p53 expression, TPS, or CPS. However, NY-ESO-1 expression was significantly associated with high $CD8^+$ T-cell infiltration in surgical specimens ($P = 0.0194$).

No significant association was observed between NY-ESO-1 expression and tertiary lymphoid structure (TLS) density ($P = 0.129$).

Associations between tumor-related biomarkers and response to nivolumab

The associations between biomarker expression and response to nivolumab are shown in Table 1. Because MLH1 loss was observed in only one patient, this marker was not included in further response analyses. No significant association was observed between p53 expression and treatment response. In contrast, NY-ESO-1 expression was significantly associated with response to nivolumab. The response rate was significantly higher in patients with NY-ESO-1–positive tumors than in those with NY-ESO-1–negative tumors (28.6% vs. 11.1%, $P = 0.005$). PD-L1 expression was also associated with treatment response. Patients with CPS ≥ 10% had a significantly higher response rate than those with CPS < 10% (24.1% vs. 10.2%, $P = 0.003$). Similarly, patients with TPS ≥ 1% showed a higher response rate than those with TPS < 1% (20.3% vs. 9.4%, $P = 0.018$). Univariate analysis of factors associated with response to nivolumab demonstrated significant associations with NY-ESO-1 expression, TPS score, and CPS score (Table 2). In multivariate analysis, NY-ESO-1 expression remained an independent factor associated with response to nivolumab (OR 3.32, 95% CI 1.49–7.28, $P = 0.0027$), whereas TPS and CPS scores were not retained in the final model.

Associations between tumor-related biomarkers and survival after nivolumab treatment

Kaplan–Meier survival analyses according to biomarker expression are shown in Fig. 3. p53 expression was not associated with survival outcomes. Median progression-free survival (PFS) was 2.5 months in p53-positive tumors and 2.1 months in p53-negative tumors ($P = 0.807$), while median overall survival (OS) was 11.1 and 10.8 months, respectively ($P = 0.608$). NY-ESO-1 expression was not significantly associated with OS (11.2 vs. 10.6 months, $P = 0.470$), although a trend toward longer PFS was observed in NY-ESO-1–positive tumors (3.4 vs. 2.2 months, $P = 0.0718$). PD-L1 TPS was not significantly associated with survival outcomes. Median PFS was 2.5 months in patients with TPS ≥ 1% and 2.0 months in those with TPS < 1% ($P = 0.131$), while median OS was 10.9 months in both groups ($P = 0.188$). In contrast, CPS was significantly associated with survival. Patients with CPS ≥ 10% had significantly longer median PFS (2.6 vs. 2.0 months, $P = 0.0173$) and OS (12.3 vs. 10.4 months, $P = 0.0479$) than those with CPS < 10%.

Univariate analysis of factors associated with PFS identified performance status, the number of metastatic organs, and CPS score as factors significantly associated with PFS (Table 3). Multivariate analysis further identified performance status (HR 1.37, 95% CI 1.05–1.81, $P = 0.0209$) and the number of metastatic organs (HR 1.53, 95% CI 1.08–2.18, $P = 0.0158$) to be independent prognostic factors. CPS score showed a marginal association with PFS (HR 1.33, 95% CI 0.98–1.80, $P = 0.0603$).

Exploratory combined analysis of tumor-related and host immune factors associated with patient survival

Exploratory combined analyses incorporating previously evaluated host immune microenvironment factors were performed in patients with surgical specimens (Fig. 4). Patients with TLS-high/NY-ESO-1–positive tumors (n = 14) showed significantly better PFS (NR vs. 2.3 months, $P = 0.0025$) and OS (NR vs. 10.9 months, $P = 0.0046$) than the remaining patients (n = 115). Similarly, patients with CD8-high/NY-ESO-1–positive tumors (n = 16) had significantly longer PFS (6.5 vs. 2.5 months, $P = 0.0236$) and showed a trend toward improved OS (NR vs. 11.2 months, $P = 0.0512$) compared with the remaining patients (n = 113). Furthermore, patients with NY-ESO-1–positive tumors and CPS ≥ 10% (n = 16) demonstrated significantly longer PFS (4.4 vs. 2.3 months, $P = 0.0167$) and OS (20.6 vs. 10.4 months, $P = 0.0194$) than the other patients (n = 234). Because these favorable subgroups were small, these findings should be interpreted as exploratory observations.

Discussion

In this multicenter biomarker study of patients with unresectable or recurrent esophageal squamous cell carcinoma treated with nivolumab, we identified NY-ESO-1 expression as a tumor-related factor significantly associated with treatment response. In addition, PD-L1 expression, particularly when assessed using the combined positive score (CPS), was associated with both response and survival outcomes. In contrast, p53 expression showed no clear association with nivolumab efficacy, and MLH1 loss was extremely rare in this cohort. Furthermore, exploratory integration with host immune factors suggested additional prognostic heterogeneity. Collectively, these findings suggest that tumor antigenicity represented by NY-ESO-1 and immune contexture reflected by PD-L1 and host immune parameters may jointly influence the clinical benefit of PD-1 blockade in ESCC.

NY-ESO-1 is one of the most immunogenic cancer-testis antigens and is capable of eliciting both humoral and cellular

Table 3 Univariate and multivariate analyses of factors associated with progression-free survival

Variables	Category	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Age (y)	≤70	0.95 (0.73–1.24)	0.724		
Sex	Female	1.06 (0.77–1.45)	0.713		
Performance status	1–3	1.36 (1.04–1.78)	**0.0221**	1.37 (1.05–1.81)	**0.0209**
History of smoking	Yes	0.94 (0.70–1.29)	0.739		
Previous surgery	No	1.27 (0.97–1.66)	0.0792	1.11 (0.84–1.47)	0.442
Previous radiotherapy	Yes	0.87 (0.66–1.14)	0.3230		
Number of previous chemotherapy regimens	3-	0.96 (0.67–1.38)	0.822		
Number of organs with metastases	3-	1.61 (1.14–2.28)	**0.0067**	1.53 (1.08–2.18)	**0.0158**
p53	Yes	1.04 (0.78–1.39)	0.750		
NY-ESO-1	No	1.37 (0.97–1.94)	0.0735	1.34 (0.94–1.93)	0.1033
TPS	<1%	1.20 (0.92–1.57)	0.177		
CPS	<10%	1.38 (1.04–1.85)	**0.0274**	1.33 (0.98–1.80)	0.0603

CI confidence interval, *HR* hazard ratio, *PD-L1* programmed death-ligand 1, *TPS* tumor proportion score, *CPS* combined positive score

immune responses in cancer patients [32, 33]. Previous studies have demonstrated NY-ESO-1 expression and immunogenicity in esophageal cancer, supporting its biological relevance as a tumor-associated antigen in this disease [33]. Prior studies suggest a possible biological rationale linking NY-ESO-1-related immunity to PD-1 blockade, but the present study does not provide direct mechanistic evidence [34].

In our cohort, NY-ESO-1–positive tumors were significantly associated with higher $CD8^+$ T-cell infiltration in surgical specimens, suggesting that NY-ESO-1 expression may reflect an immune-reactive tumor phenotype with preexisting antitumor immunity. These observations are also supported by prior clinical studies demonstrating that NY-ESO-1–specific immune responses correlate with clinical benefit from immune checkpoint blockade and that NY-ESO-1–directed immune strategies remain biologically relevant in esophageal cancer [35, 36]. The lack of a significant association with overall survival despite the response signal may reflect limited statistical power and the small number of responders in the NY-ESO-1-positive subgroup. Although post-progression treatment did not significantly differ according to NY-ESO-1 status (19/49 [38.8%] vs. 104/201 [51.7%], $P = 0.102$), subsequent therapies after nivolumab may still have influenced overall survival.

PD-L1 expression was also associated with response when evaluated by both TPS and CPS, and a high CPS was linked to favorable progression-free and overall survival in our cohort. These findings are broadly consistent with previous studies in ESCC indicating that PD-L1 expression, particularly when assessed using CPS, is associated with improved benefit from nivolumab, although its predictive performance as a single biomarker remains limited [37, 38]. A methodological strength of the present study is that TPS and CPS were quantified objectively using the automated digital image analysis platform HALO. This approach is relevant because PD-L1 scoring, especially CPS, is known to show substantial interobserver variability due to the difficulty of consistently identifying PD-L1–positive immune cells in addition to tumor cells [39, 40]. Automated whole-slide analysis has been suggested to improve reproducibility and reduce observer-dependent variability in PD-L1 assessment [41, 42]. Nevertheless, PD-L1 did not retain independent significance in the multivariate model, indicating that PD-L1 alone may not fully capture sensitivity to PD-1 blockade. Collectively, these findings suggest that PD-L1 remains a clinically relevant but incomplete biomarker, and that its utility may be enhanced when interpreted in combination with other tumor-related or host immune microenvironment factors.

In the present cohort, NY-ESO-1 expression was not significantly associated with PD-L1 TPS or CPS, but was significantly associated with high $CD8^+$ T-cell infiltration in surgical specimens. These findings suggest that NY-ESO-1 and PD-L1 capture related but nonredundant aspects of

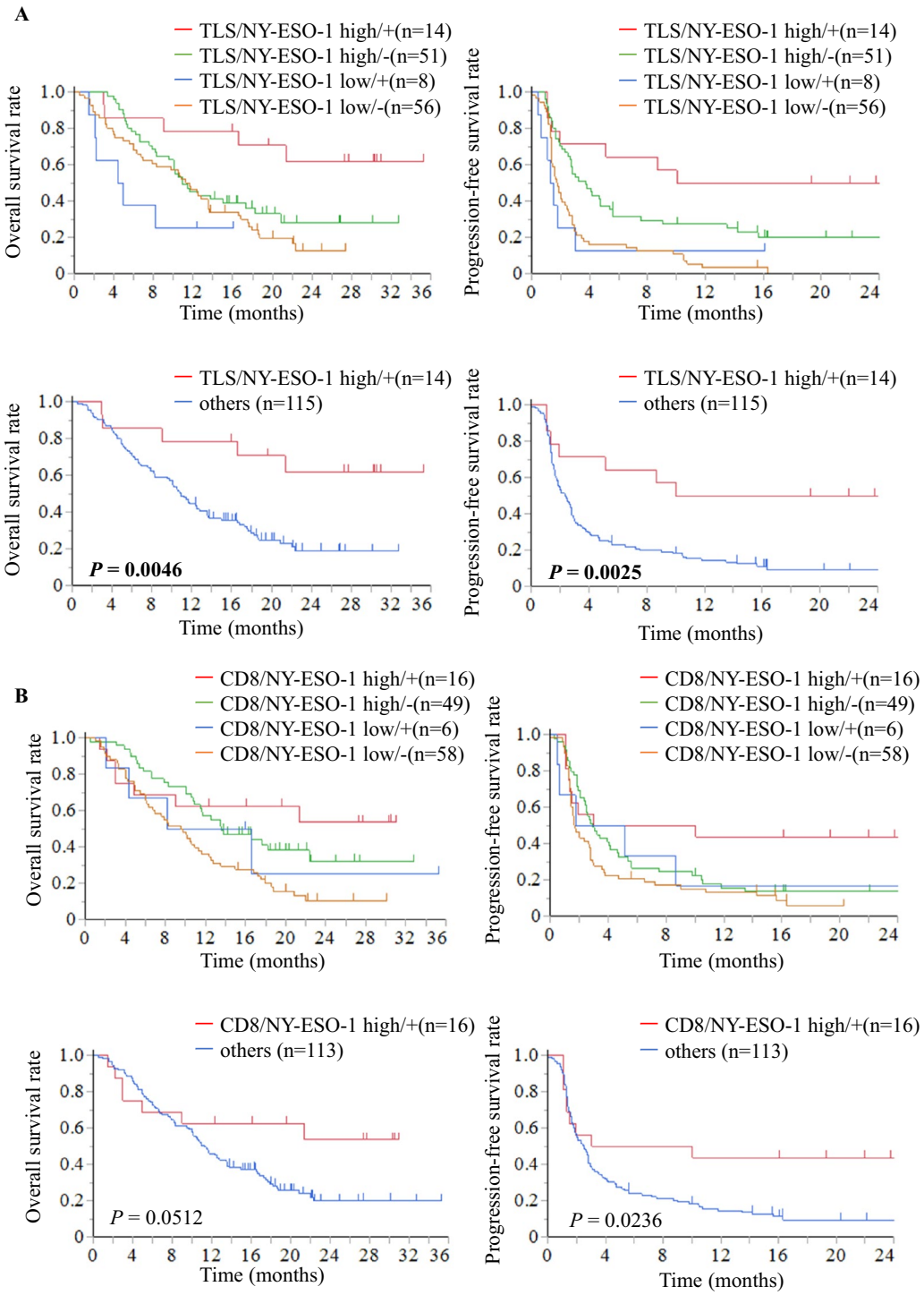


Fig. 4 Exploratory combined survival analyses of tumor-related and host immune microenvironment factors in surgical specimens. **A** OS and PFS according to combined TLS density and NY-ESO-1 expression. **B** OS and PFS according to combined $CD8^+$ cell infiltration and NY-ESO-1 expression. **C** OS and PFS according to combined NY-ESO-1 expression and PD-L1 CPS. Patients with TLS-high/NY-ESO-1–positive tumors, CD8-high/NY-ESO-1–positive tumors, or NY-ESO-1–positive tumors with CPS ≥ 10% tended to show more favorable survival outcomes than the remaining patients; however, these findings should be interpreted cautiously because the favorable subgroups were small. The host immune microenvironment factors used in these combined analyses were previously evaluated in the same cohort (PMID: 40,274,705). Representative images and a schematic summary of the quantification methods for these host immune microenvironment factors are provided in Supplementary Figure S3 (PMID: 40274705)

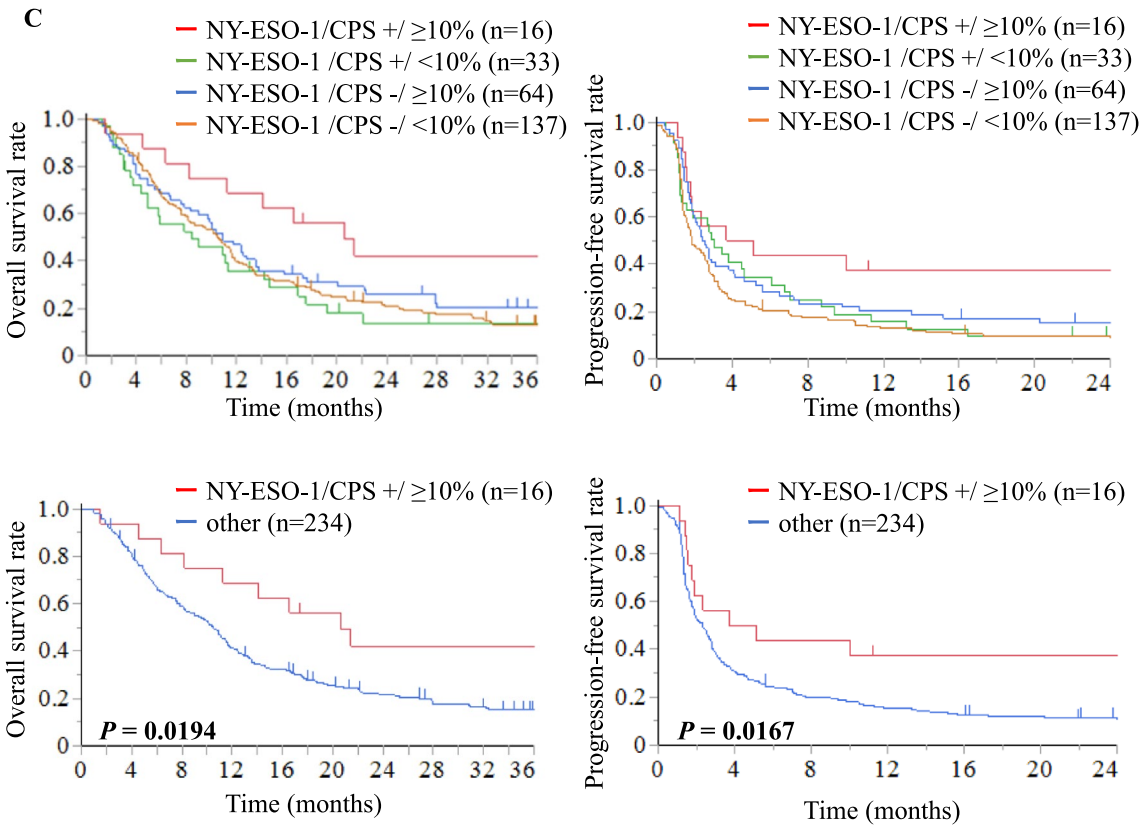


Fig. 4 (continued)

tumor-immune biology and may partly explain why NY-ESO-1, but not PD-L1, remained independently associated with response in the multivariate model. Therefore, NY-ESO-1 may complement PD-L1 rather than replace it as a biomarker in nivolumab-treated ESCC. Because both markers may also reflect general tumor immunogenicity or prognosis in non-ICI settings, the present findings should be interpreted as associations within a nivolumab-treated cohort rather than as definitive evidence of treatment-specific predictive biomarkers.

Our results also suggest that evaluation of a single tumor-related factor may be insufficient to fully capture sensitivity to PD-1 blockade. In our cohort, NY-ESO-1 expression was significantly associated with increased $CD8^+$ T-cell infiltration in surgical specimens, supporting the biological plausibility of our combined analyses. NY-ESO-1 is a highly immunogenic cancer-testis antigen capable of eliciting spontaneous cellular and humoral immune responses in cancer patients, and previous studies have demonstrated NY-ESO-1–specific $CD8^+$ T-cell responses in esophageal cancer [32, 33]. Moreover, PD-1 signaling has been shown to regulate the expansion of NY-ESO-1–specific $CD8^+$ T cells, providing a mechanistic rationale linking NY-ESO-1–related immunity to PD-1 blockade [34]. Consistent with this concept, patients with TLS-high/NY-ESO-1-positive or CD8-high/NY-ESO-1-positive tumors showed more favorable survival outcomes than the remaining patients in our cohort. Although direct evidence linking NY-ESO-1 expression to TLS formation in ESCC remains limited, TLS-rich esophageal tumors have been associated with PD-1–responsive $CD8^+$ T-cell populations, and studies in other tumor types have also linked NY-ESO-1 expression with TLS-positive immune microenvironments [43, 44]. However, these small-subgroup analyses should be considered exploratory and hypothesis-generating. Collectively, these findings suggest that tumor antigenicity represented by NY-ESO-1 and host immune contexture reflected by $CD8^+$ T-cell infiltration or TLS may provide complementary information for exploratory stratification in nivolumab-treated ESCC.

This study has several limitations. First, its retrospective multicenter design means that treatment strategies, imaging assessments, and follow-up schedules were not fully standardized across institutions. However, consecutive patients treated with nivolumab were collected at each participating center, which may have reduced selection bias. Second, the analyzed archival primary tumor specimens may not fully reflect tumor biology or the immune microenvironment at nivolumab initiation. Because nivolumab

was given as second-line or later therapy, most patients had received prior systemic treatment and/or radiotherapy, and paired archival and immediate pre-nivolumab specimens were not available. PD-L1 expression is known to vary according to prior treatment and spatial heterogeneity [45–47]. Treatment exposure between tissue acquisition and nivolumab initiation did not significantly differ according to NY-ESO-1 status (Supplementary Table S3). However, temporal mismatch may still have introduced biomarker misclassification, particularly for dynamic markers such as PD-L1, CD8 + cell infiltration, and potentially NY-ESO-1. Third, most analyses were based on primary tumor specimens, and therefore biomarker expression in metastatic or recurrent lesions could not be evaluated. Fourth, although HALO-based digital image analysis improved the objectivity of biomarker quantification, the predefined cutoff values remain exploratory. In addition, the present findings were derived from a single retrospective cohort without external validation, and the association between NY-ESO-1 expression and treatment response should be interpreted cautiously. In particular, p53 immunoreactivity does not necessarily represent the full spectrum of TP53 alterations, and MLH1 loss was extremely rare in our cohort [48]. Finally, the combined analyses were exploratory and require validation in independent cohorts.

In conclusion, this multicenter biomarker study evaluated tumor-related factors using automated digital image analysis in a large cohort of patients with unresectable or recurrent ESCC treated with nivolumab. NY-ESO-1 expression was associated with response to nivolumab, while PD-L1 expression, particularly CPS, was associated with clinical outcomes. These findings suggest that integrated evaluation of tumor antigenicity and host immune contexture may aid exploratory stratification of nivolumab-treated ESCC, although external validation is warranted.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00262-026-04415-x.

Acknowledgements The authors thank the staff of the following centers for their participation in data collection: Department of Surgery, Rinku General Medical Center; Department of Surgery, JCHO Osaka Hospital; Department of Gastrointestinal Surgery, Kansai Rosai Hospital; Department of Surgery, Hyogo Prefectural Nishinomiya Hospital; Department of Surgery, Toyonaka Municipal Hospital; Department of Surgery, Yao Municipal Hospital; Department of Surgery, Minoh City Hospital; Department of Gastrointestinal Oncology, Osaka International Cancer Institute; Department of Surgery, Osaka General Medical Center; Department of Gastroenterological Surgery, Kindai University Nara Hospital; Department of Surgery, Sakai City Medical Center; Department of Surgery, National Hospital Organization Osaka National Hospital; Department of Gastroenterological Surgery, Higashiosaka City Medical Center; and Department of Surgery, Osaka Rosai Hospital.

Author contributions S.N.: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing—original draft. T.M.: Conceptualization, methodology, supervision, and writing—review and editing. K.M., K.Y., K.T., H.M., S.Y., M.M., Y.Ki., T.T., M.H., J.M., and Y.A.: Investigation, data curation, and resources. Y.Ku.: Supervision and writing—review and editing. E.M.: Pathological review, methodology, and writing—review and editing. H.E. and Y.D.: Supervision, project administration, and writing—review and editing. All authors contributed to the study design, critically revised the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

Funding Takeda Science Foundation, a Grant-in-Aid for Scientific Research (C) (21K08776, 24K11865); the Princess Takamatsu Cancer Research Fund; the Japan Research Foundation for Clinical Pharmacology; and the Uehara Memorial Foundation.

Data availability The data generated in this study are available upon request from the corresponding author.

Declarations

Conflict of interest T.M. has received lecture fees from Ono Pharmaceutical Co., Ltd. and Bristol Myers Squibb. Y.Ku. has received lecture fees and research grants from Ono Pharmaceutical Co., Ltd. and lecture fees from Bristol Myers Squibb. Y.D. has received lecture fees and research grants from Ono Pharmaceutical Co., Ltd. and lecture fees from Bristol Myers Squibb. All other authors declare no competing interests.

Consent to participate Written informed consent was obtained from all included patients before enrollment, unless the patient had died or been lost to follow-up.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the institutional review boards of all participating institutions (approval no. 19146-5).

References

1. Qi L, Sun M, Liu W et al (2024) Global esophageal cancer epidemiology in 2022 and predictions for 2050: a comprehensive analysis and projections based on GLOBOCAN data. Chin Med J

(Engl) 137:3108–3116. https://doi.org/10.1097/CM9.0000000000003420
2. Morgan E, Soerjomataram I, Rumgay H et al (2022) The global landscape of esophageal squamous cell carcinoma and esophageal adenocarcinoma incidence and mortality in 2020 and projections to 2040: new estimates from GLOBOCAN 2020. Gastroenterology 163:649-658.e2. https://doi.org/10.1053/j.gastro.2022.05.054
3. Deboever N, Jones CM, Yamashita K et al (2024) Advances in diagnosis and management of cancer of the esophagus. BMJ 385:e074962. https://doi.org/10.1136/bmj-2023-074962
4. Kubo Y, Igaue S, Utsunomiya D et al (2024) Association between preoperative serum zinc level and prognosis in patients with advanced esophageal cancer in the neoadjuvant treatment era. Ann Gastroenterol Surg 8:595–603. https://doi.org/10.1002/ags3.12781
5. Matsumoto C, Iwatsuki M, Mitsuura C et al (2024) Textbook outcome contributes to long-term prognosis in elderly patients with esophageal squamous cell carcinoma. Ann Gastroenterol Surg 8:787–794. https://doi.org/10.1002/ags3.12799
6. Makino T, Yamasaki M, Tanaka K et al (2022) Multicenter randomised trial of two versus three courses of preoperative cisplatin and fluorouracil plus docetaxel for locally advanced oesophageal squamous cell carcinoma. Br J Cancer 126:1555–1562. https://doi.org/10.1038/s41416-022-01726-5
7. Nose Y, Makino T, Tatsumi M et al (2023) Risk stratification of oesophageal squamous cell carcinoma using change in total lesion glycolysis and number of PET-positive lymph nodes. Br J Cancer 128:1879–1887. https://doi.org/10.1038/s41416-023-02151-y
8. Hashimoto T, Makino T, Yamasaki M et al (2020) The pattern of residual tumor after neoadjuvant chemotherapy for locally advanced esophageal cancer and its clinical significance. Ann Surg 271:875–884. https://doi.org/10.1097/SLA.0000000000003129
9. Makino T, Yamasaki M, Tanaka K et al (2019) Metabolic tumor volume change predicts long-term survival and histological response to preoperative chemotherapy in locally advanced esophageal cancer. Ann Surg 270:1090–1095. https://doi.org/10.1097/SLA.0000000000002808
10. Makino T, Nakai S, Momose K et al (2024) Efficacy and survival of nivolumab treatment for recurrent/unresectable esophageal squamous-cell carcinoma: real-world clinical data from a large multi-institutional cohort. Esophagus 21:319–327. https://doi.org/10.1007/s10388-024-01056-w
11. Makino T, Nakai S, Hagi T et al (2026) Conversion surgery following immunochemotherapy for initially unresectable/recurrent esophageal cancer. Esophagus 23:131–138. https://doi.org/10.1007/s10388-025-01176-x
12. Javed SA, Najmi A, Ahsan W, Zoghebi K (2024) Targeting PD-1/PD-L1 immune checkpoint inhibition for cancer immunotherapy: success and challenges. Front Immunol 15:1383456. https://doi.org/10.3389/fimmu.2024.1383456
13. Kato K, Cho BC, Takahashi M et al (2019) Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 20:1506–1517. https://doi.org/10.1016/S1470-2045(19)30626-6
14. Okada M, Kato K, Cho BC et al (2022) Three-year follow-up and response-survival relationship of nivolumab in previously treated patients with advanced esophageal squamous cell carcinoma (ATTRACTION-3). Clin Cancer Res 28:3277–3286. https://doi.org/10.1158/1078-0432.CCR-21-0985
15. Sun M, Yang P, Wang W (2024) Advancements in the research of immune checkpoint inhibitors for the treatment of advanced esophageal squamous cell carcinoma. Am J Cancer Res 14:1981–1998. https://doi.org/10.62347/XUWC6412
16. Wang Z, Zhang RY, Xu YF et al (2025) Unmasking immune checkpoint resistance in esophageal squamous cell carcinoma: insights into the tumor microenvironment and biomarker landscape. World J Gastrointest Oncol 17:109489. https://doi.org/10.4251/wjgo.v17.i8.109489
17. Matsubara Y, Toriyama K, Kadowaki S et al (2023) The impact of combined PD-L1 positive score on clinical response to nivolumab in patients with advanced esophageal squamous cell carcinoma. Esophagus 20:524–532. https://doi.org/10.1007/s10388-022-00978-7
18. Chi Z, Peng L, Karamchandani DM et al (2025) PD-L1 (22C3) expression and prognostic implications in esophageal squamous cell carcinoma. Ann Diagn Pathol 74:152394. https://doi.org/10.1016/j.anndiagpath.2024.152394
19. Le DT, Durham JN, Smith KN et al (2017) Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357:409–413. https://doi.org/10.1126/science.aan6733
20. Hagi T, Kurokawa Y, Kawabata R et al (2020) Multicentre biomarker cohort study on the efficacy of nivolumab treatment for gastric cancer. Br J Cancer 123:965–972. https://doi.org/10.1038/s41416-020-0975-7
21. Kurose K, Sakaeda K, Fukuda M et al (2023) Immune checkpoint therapy and response biomarkers in non-small-cell lung cancer: serum NY-ESO-1 and XAGE1 antibody as predictive and monitoring markers. Adv Clin Chem 112:155–204. https://doi.org/10.1016/bs.acc.2022.09.004
22. Jung EH, Jang HR, Kim SH et al (2021) Tumor LAG-3 and NY-ESO-1 expression predict durable clinical benefits of immune checkpoint inhibitors in advanced non-small cell lung cancer. Thorac Cancer 12:2237–2245. https://doi.org/10.1111/1759-7714.13834
23. Zhong L, Li H, Chang W et al (2023) TP53 mutations in esophageal squamous cell carcinoma. Front Biosci (Landmark Ed) 28:193. https://doi.org/10.31083/j.fbl2809219
24. Jin Y, Zhao X, Song X et al (2023) The high expression of p53 is predictive of poor survival rather than TP53 mutation in esophageal squamous cell carcinoma. J Oncol 2023:3801526. https://doi.org/10.1155/2023/3801526
25. Nakai S, Makino T, Momose K et al (2025) Exploring predictive biomarkers of efficacy and survival with nivolumab treatment for unresectable/recurrent esophageal squamous cell carcinoma. Esophagus 22:360–372. https://doi.org/10.1007/s10388-025-01120-z
26. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247. https://doi.org/10.1016/j.ejca.2008.10.026
27. Urakawa S, Makino T, Yamasaki M et al (2021) Lymph node response to neoadjuvant chemotherapy as an independent prognostic factor in metastatic esophageal cancer. Ann Surg 273:1141–1149. https://doi.org/10.1097/SLA.0000000000003445
28. Hagi T, Makino T, Yamasaki M et al (2022) Pathological regression of lymph nodes better predicts long-term survival in esophageal cancer patients undergoing neoadjuvant chemotherapy followed by surgery. Ann Surg 275:1121–1129. https://doi.org/10.1097/SLA.0000000000004238
29. Noma T, Makino T, Ohshima K et al (2023) Immunoscore signatures in surgical specimens and tumor-infiltrating lymphocytes in pretreatment biopsy predict treatment efficacy and survival in esophageal cancer. Ann Surg 277:528–537. https://doi.org/10.1097/SLA.0000000000005104
30. Doki Y, Ajani JA, Kato K et al (2022) Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. N Engl J Med 386:449–462. https://doi.org/10.1056/NEJMoa2111380
31. Hayashi Y, Makino T, Sato E et al (2023) Density and maturity of peritumoral tertiary lymphoid structures in oesophageal squamous

cell carcinoma predicts patient survival and response to immune checkpoint inhibitors. Br J Cancer 128:2175–2185. https://doi.org/10.1038/s41416-023-02235-9
32. Gnjatic S, Nishikawa H, Jungbluth AA et al (2006) NY-ESO-1: review of an immunogenic tumor antigen. Adv Cancer Res 95:1–30. https://doi.org/10.1016/S0065-230X(06)95001-5
33. Fujita S, Wada H, Jungbluth AA et al (2004) NY-ESO-1 expression and immunogenicity in esophageal cancer. Clin Cancer Res 10:6551–6558. https://doi.org/10.1158/1078-0432.CCR-04-0819
34. Fourcade J, Kudela P, Andrade Filho PA et al (2009) PD-1 is a regulator of NY-ESO-1-specific CD8+ T cell expansion in melanoma patients. J Immunol 182:5240–5249. https://doi.org/10.4049/jimmunol.0803245
35. Yuan J, Adamow M, Ginsberg BA et al (2011) Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. Proc Natl Acad Sci U S A 108:16723–16728. https://doi.org/10.1073/pnas.1110814108
36. Ishikawa T, Kageyama S, Miyahara Y et al (2021) Safety and antibody immune response of CHP-NY-ESO-1 vaccine combined with poly-ICLC in advanced or recurrent esophageal cancer patients. Cancer Immunol Immunother 70:3081–3091. https://doi.org/10.1007/s00262-021-02892-w
37. Lee J, Kim B, Jung HA et al (2021) Nivolumab for esophageal squamous cell carcinoma and the predictive role of PD-L1 or CD8 expression in its therapeutic effect. Cancer Immunol Immunother 70:1203–1211. https://doi.org/10.1007/s00262-020-02766-7
38. Wang Y, Abu-Sbeih H, Mao E et al (2022) Association of PD-L1 expression and other variables with benefit from immune checkpoint inhibition in advanced gastroesophageal cancer: systematic review and meta-analysis of 17 phase 3 randomized clinical trials. JAMA Oncol 8:1635–1643. https://doi.org/10.1001/jamaoncol.2022.3707
39. Robert ME, Rüschoff J, Jasani B et al (2023) High interobserver variability among pathologists using combined positive score to evaluate PD-L1 expression in gastric, gastroesophageal junction, and esophageal adenocarcinoma. Mod Pathol 36:100154. https://doi.org/10.1016/j.modpat.2023.100154
40. Mercier A, Conan-Charlet V, Quintin-Roué I et al (2023) Reproducibility in PD-L1 immunohistochemistry quantification through the tumor proportion score and the combined positive score: could dual immunostaining help pathologists? Cancers (Basel) 15:2768. https://doi.org/10.3390/cancers15102768
41. Taylor CR, Jadhav AP, Gholap A et al (2019) A multi-institutional study to evaluate automated whole slide scoring of immunohistochemistry for assessment of programmed death-ligand 1 (PD-L1) expression in non-small cell lung cancer. Appl Immunohistochem Mol Morphol 27:263–269. https://doi.org/10.1097/PAI.0000000000000737
42. Toivonen S, Rokka A, Ostromohov N et al (2022) Development and validation of a supervised deep learning algorithm for automated whole-slide programmed death-ligand 1 tumour proportion score assessment in non-small cell lung cancer. Histopathology 80:693–705. https://doi.org/10.1111/his.14571
43. Tanoue K, Ohmura H, Uehara K et al (2024) Spatial dynamics of CD39+CD8+ exhausted T cell reveal tertiary lymphoid structures-mediated response to PD-1 blockade in esophageal cancer. Nat Commun 15:9033. https://doi.org/10.1038/s41467-024-53262-w
44. Magara T, Nakamura M, Nojiri Y et al (2023) Tumor immune microenvironment of cutaneous angiosarcoma with cancer testis antigens and the formation of tertiary lymphoid structures. Front Oncol 13:1106434. https://doi.org/10.3389/fonc.2023.1106434
45. Okadome K, Baba Y, Yasuda-Yoshihara N et al (2022) PD-L1 and PD-L2 expression status in relation to chemotherapy in primary and metastatic esophageal squamous cell carcinoma. Cancer Sci 113:399–410. https://doi.org/10.1111/cas.15198
46. Zou Y, Hu X, Zheng S et al (2021) Discordance of immunotherapy response predictive biomarkers between primary lesions and paired metastases in tumours: a systematic review and meta-analysis. EBioMedicine 63:103137. https://doi.org/10.1016/j.ebiom.2020.103137
47. Yu B, Qi C, Liu Z et al (2025) Spatial heterogeneity of PD-L1 expression influence its assessment in esophageal squamous cell carcinoma. Transl Oncol 59:102442. https://doi.org/10.1016/j.tranon.2025.102442
48. Niyaz M, Ainiwaer J, Abudureheman A et al (2020) Association between TP53 gene deletion and protein expression in esophageal squamous cell carcinoma and its prognostic significance. Oncol Lett 20:1855–1865. https://doi.org/10.3892/ol.2020.11709